Acid Deposition in Madrid and Corticolous Myxomycetes

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Abstract

This paper presents the results of a series of simultaneous moist chamber cultures which were set up using the bark of living *Quercus ilex* as a substrate for corticolous myxomycetes. Samples of bark were taken within the same 48 hour period from zones of Madrid with different levels of urban atmospheric contamination. The areas sampled were to the West (upwind), to the East (downwind) and in the centre of the city of Madrid. The quantities of atmospheric pollutants recorded for the same period by permanent monitoring stations from Madrid Community showed an inverse relationship with the number of myxomycetes recorded, and the variety of species was also greater in the unpolluted West. The species most affected were the typical corticoles. Data on bark pH suggest that there is still considerable buffering taking place in the bark from this area, which means that the actual effect of acidification on myxomycetes is probably even greater. The large scale study of myxomycete abundance and diversity compared to existing acid precipitation and forest damage data is urged, and their use, in addition to lichens, and bark itself as bioindicators of forest health, is suggested.

Key Words

Corticolous myxomycetes, pH, acid precipitation, Madrid, bioindicator.

Stapfia 73,
zugleich Kataloge des OÖ. Landesmuseums,
Neue Folge Nr. 155 (2000), 113-120.

Introduction

The direct effects of acid precipitation on corticolous epiphytes such as the lichens, are already well documented, and these organisms have been frequently used as air pollution monitors, especially in the case of sulphur dioxide levels (HALLINGBÄCK 1989; BATES et al. 1990; SEAWARD 1992; RICHARDSON 1992), but very little research has been carried out to see if this is also true for the myxomycetes. Distribution studies have shown that trees with less acidic and rougher bark such as species of *Juniperus*, *Ulmus* and *Quercus* (BROOKS et al. 1977) are better substrates for the growth of myxomycetes, and some studies by investigators sampling trees in acid woods and woods of lower acid deposition have already shown that distribution of myxomycetes may be affected by acid deposition (HÄRKÖNEN 1977, 1978; STEPHENSON 1988, 1989). STEPHENSON (1989) and ING (1994) made reference to the apparent importance of pH in determining distribution patterns of myxomycetes.

The purpose of this paper was an initial attempt to find out if there is any difference in the corticolous myxomycete distribution along an urban pollution gradient, in zones of varied atmospheric contamination across the city of Madrid, which might suggest the relative sensitivity of myxomycetes to acid deposition.

Acid precipitation as an immediate cause of visible tree damage is still a disputed topic, and the precise nature of its damaging effects, recognised for more than a century (COWLING 1982), is still controversial. Although it is professionally agreed that wet and dry deposition of acid have harmful effects on forest ecosystems, confusing, misrepresented and often erroneous reports (EHRLICH & EHRLICH 1996) have surrounded the issue and lead to the illusion of lack of scientific consensus. The complexities of the atmospheric chemistry causing and ameliorating acid deposition are being re-examined (LIKENS et al. 1996; HEDIN & LIKENS 1996) and suggest that that the pollution damage to forest systems may be even more serious than previously expected. Current research (ASHMORE et al. 1990; DOE 1993; APSIMON et al. 1997) supposes that much of the forest damage caused by anthropogenic atmospheric pollution is probably due to a combination of factors which upset the normal interspecific relationships among forest inhabitants, rather than any direct effect of the acid on the trees. There is also evidence that pest populations of trees, and fungal attack, increase with higher levels of acid deposition, but no research has been done to gauge the effect of this pollution on the predators and parasites of these pests, and some effects diagnosed as multiple stress have been found to be due to one agent (APSIMON et al. 1997).

The myxomycetes are an important component of the corticolous microhabitat (ING 1994), and many species are exclusive to this particular habitat. They have been collected on most types of living trees where they complete the trophic stages of their life cycle, mainly as phagotrophic bacterivores. Their spores land on the bark of all species of trees carried by wind, local air currents, arthropod and probably other vectors (STEPHENSON & STEMPEN 1994; ING 1994). Myxomycetes are voracious feeders of bacteria, fungal spores and fungal hyphae (MARTIN & ALEXOPOULOS 1969; STEPHENSON & STEMPEN 1994) both in their plasmodial and unicellular stages. CLARHOLM (1981) suggested that grazing by all naked amoebae in soil indicated a role for them as bacterial regulators. She reported that amoebae were responsible for 60% of bacterial decrease. FEEST & MADELIN (1988) found that myxomycetes made up some 50% of all soil amoebae in their study, and the authors estimated that their predation could account for a significant amount of bacterial decline and play an important role in detritus food chains. STEPHENSON & CAVENDER (1996) also suggested that a significant percentage of naked amoebae is made up of myxomonads, and JACOBSON (1980) reported that one myxamoeba eats 200 bacteria in a single cell doubling time. Maintenance of the considerable biomass of the plasmodia, especially of some larger species, will require even greater predation. Disturbances to such a crucial component of the corticolous biota must affect the species balance of other corticolous inhabitants.

Materials and Methods

A series of simultaneous moist chamber cultures were set up, from four zones of Madrid (Table 1) with different levels of atmospheric contamination, using *Quercus ilex* which is one of the most characteristic and abundant trees of the Mediterranean region, as a substrate. The areas sampled were to the West (upwind), to the East (downwind) and in the centre of the city of Madrid (Fig. 1). The city is at an altitude of 700 m with hot dry summers, cold dry winters and an annual rainfall of about 600 mm. The areas were chosen according to the presence of large mature evergreen oak trees in a 60 km line across the city.

Samples were initially taken between the 2nd and 4th of May 1997, and repeated 2 years later from the same trees on the 14th and 15th of March 1999. Samples of outer bark were taken from the North to North Western side of mature trees at a height of 0.5 m-1.5 m, and put in labelled envelopes. They were placed in 45 moist chamber cultures following the same procedure described in an earlier paper (WRIGLEY de BASANTA 1998). The bark was soaked and kept moist with boiled tap water, giving a pH value of 6.8, and the pH of each culture at 24 hours (see FARMER et al. 1990) was recorded. These cultures were kept for eight weeks.

To compare the areas sampled, the term record has been used to arbitrarily denote appearances of the taxon separated by more than 5 days. This has been done to eliminate misleading comparisons between species, since in many smaller species each sporocarp is produced by an individual plasmodium, whereas

Table 1: Areas sampled for myxomycetes across Madrid. *Data from Insituto Nacional de Meterologia.

	Zone 1	Zone 2	Zone 3	Zone 4
Location of sample areas (UTM)	La Dehesa de Navalcarbon, Las Rozas (30TVK2584)	La Casa de Campo, Madrid (30 TVK3576 and 3675)	El Parque del Retiro, Madrid (30 TVK4274)	Valdelaguna (30TVK6946) Nuevo Baztán (30TVK7969) Olmeda (30TVK8068)
Altitude (m)*	725	690	667	592
Total precipitation (mm) April 1997*	48.5	55.4	40.1	26.0
February 1999*	12.5	15.7	10.5	5.0
5 year average precipitation April*	31.4	27.3	23.08	23.6
pH range of bark at 24 hrs 1997	5.7-6.2	5.7-6.1	5.5-5.7	5.5-5.9
1999	5.7-6.0	5.7-5.9	5.3-5.5	5.4-5.7
Visible epiphytes	many lichens & mosses	many lichens	few crustose lichens	acid tolerant lichens, algae or none

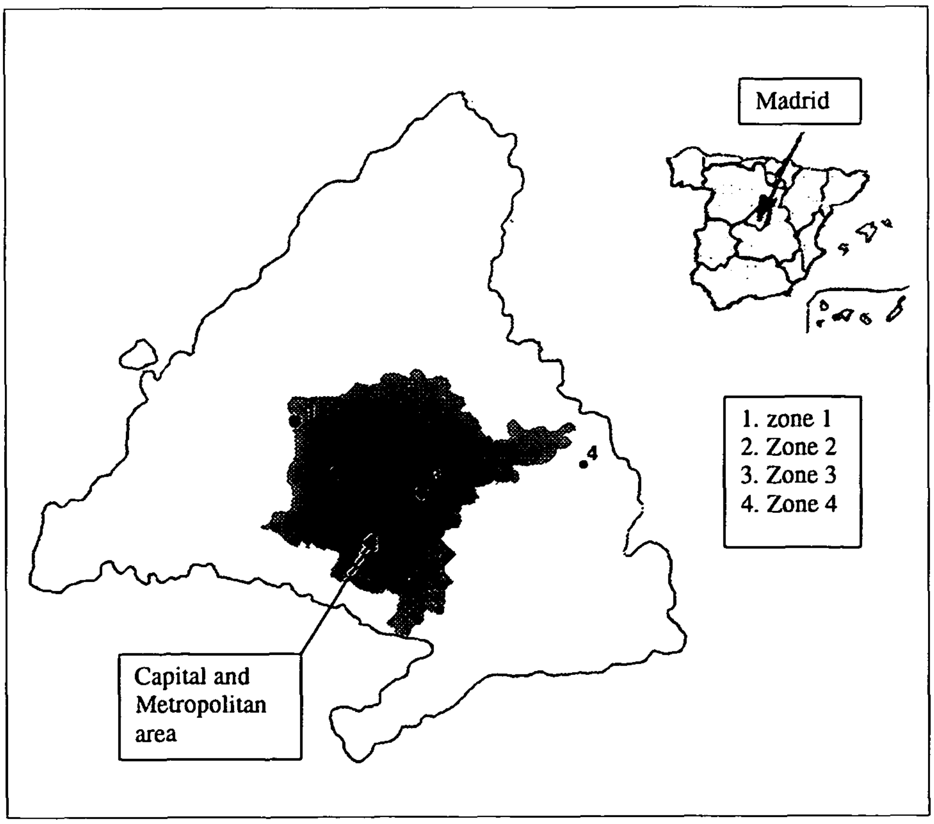

Fig. 1: Map of the sampling areas in the Province of Madrid.

other species produce various sporocarps from one plasmodium.

Data on atmospheric contaminants and rainfall were obtained from the permanent recording stations of the Community of Madrid or the city of Madrid that were closest to the sample sites. The presence of epiphytic lichens and mosses on the substrate tree bark was also noted. A diminished number of lichens shows that tree bark has undergone the effects of acid rain (RICHARDSON 1992). Observations of substrate trees in the sampling areas (Table 1) indicate that the somewhat pollution tolerant lichens like *Xanthoria parietina* and *Physcia adscendens* were the only lichen species to be found on the bark from zone 4. A total absence in zone 4 of mosses and other lichens that were present in zone 1 was an indirect indication that the bark of these trees had been subjected to acidic deposition. The rainfall data show a slight gradient from West to East in the 5 year averages, but the maximum rainfall prior to each collecting period was recorded from zone 2. The pH range of soaked bark at 24 hours also shows slight differences from West to East compared to the large difference in the quantities of oxides of nitrogen and sulphur dioxide (Fig. 2) between these areas. This is because the oak bark is buffering against acidification, although slightly less well in the later sampling. The buffering capacity of bark is reduced by continued acid deposition, as many studies have shown (BARKMAN 1958; GRODZINSKA 1977; NIEBOER et al. 1984; BATES et al. 1990).

Table 2: Myxomycete records from moist chamber cultures by zone.

Date of sampling	Zone 1 97	Zone 1 99	Zone 2 97	Zone 2 99	Zone 3 97	Zone 3 99	Zone 4 97	Zone 4 99
No. of cultures	6	6	5	6	4	6	6	6
Records by orders:								
Echinosteliales	1	0	4	1	7	11	0	0
Liceales	14	18	11	10	1	7	0	2
Physarales	2	1	1	2	4	0	9	4
Trichiales	2	1	2	10	5	8	0	0
Stemonitales	20	30	3	18	5	3	1	1
Totals each year	39	50	21	40	22	30	10	7
Total records	89		61		52		17	

Results

Mxomycetes from 5 orders were obtained from a total of 45 moist chamber cultures (Table 2). Most of the taxonomic and distribution details of the myxomycetes collected from these cultures have been reported in an earlier paper (WRIGLEY de BASANTA 1998). Zones 1 and 2 produced the most typical corticoles usually found on this substrate, and the same species that appeared in 1997 reappeared in 1999. One of the records of *Licea* in zone 4 was of a very tiny undescribed species (MITCHELL & MCHUGH 2000) which may have been missed previously. The differences in the total number of records in 1999, most notable in zone 2 (La Casa de Campo), were caused by increases in the number of small *Macbrideola* spp. particularly M. *cornea* and M. *synsporos*. A possible explanation for the increase could be that after a very dry month (Table 1) it rained on the two days before the collection date. This could have stimulated the growth of species with such a short incubation time as these *Macbrideola* spp. (WRIGLEY de BASANTA 1998). The collections from the bark from zone 4 were almost exclusively larger myxomycetes of the genus *Badhamia*, many with scant lime deposits, and one undetermined species of *Physarum*. The reasons for this are not known. They may be opportunistic species filling a niche vacated by species more susceptible to atmospheric pollutants. A large number of contaminant filamentous moulds were also observed growing in the cultures from zone 4, but not in the other cultures, despite the similar culturing conditions. Perhaps this could reflect a lack of myxomycete predation.

The data on SO^2 and NOx levels in and around the sample areas at the time of sampling (Fig. 2) have been averaged for the two collection periods and show a gradient of these pollutants from West to East. Data for zone 1 were not available as it is to the rural West of the permanently monitored region of the community, but background European data for the 2 main contaminants have been used (APSIMON et al. 1997). They show a clear West (upwind) to East (downwind) gradient for all contaminants.

An inverse relationship appears between

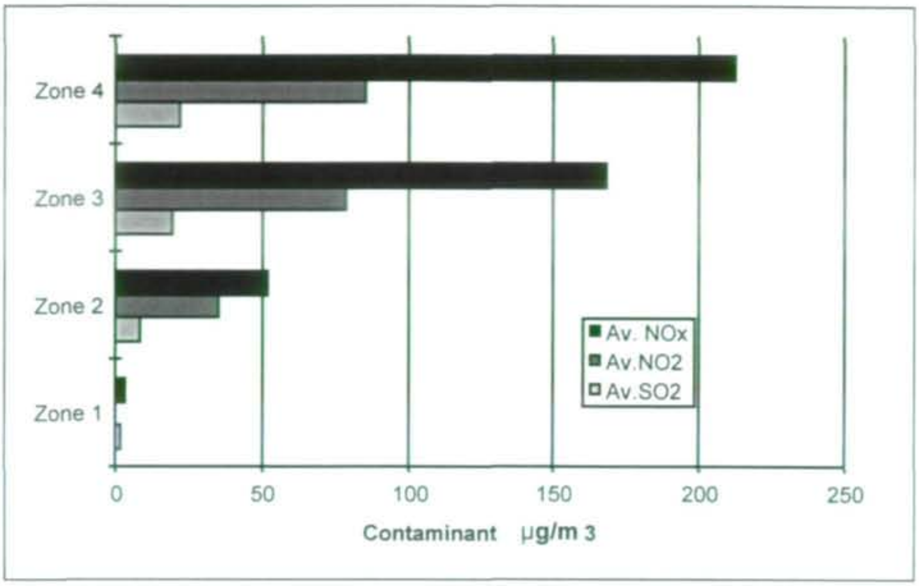

Fig. 2: Atmospheric contaminants at the four sampling zones.

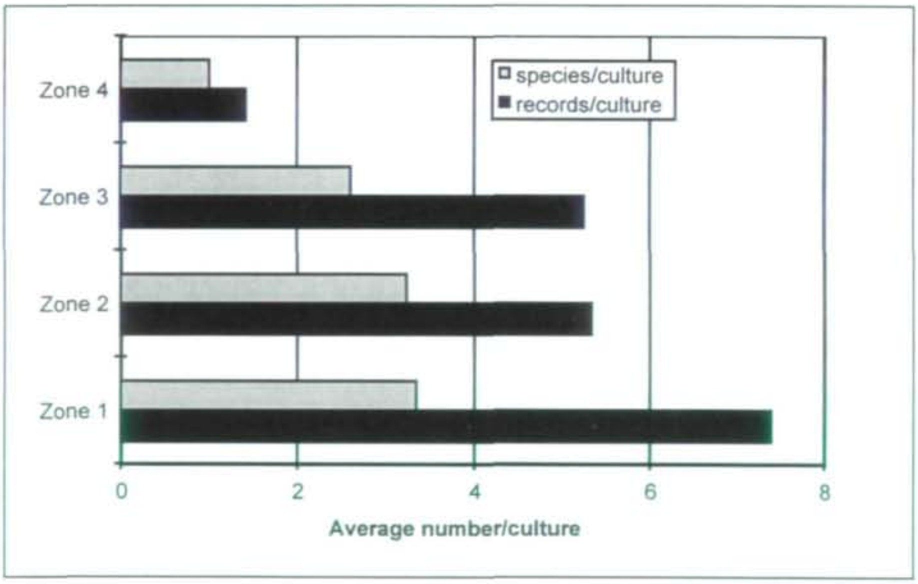

Fig. 3: Average number of myxomycetes per culture from each sampling zone.

the contaminants and the number of myxomycetes recorded, expressed as records produced per culture. The number of records of myxomycetes appearing on bark from the less contaminated zone 1 was four times greater than the number on trees in zone 4. The variety of species expressed as an average per culture, was similar from the two Westerly sites (zones 1-2) and twice as great as in zone 4 (Fig. 3).

The number of individual sporocarps produced by the most common species (Fig. 4) also follows a reverse gradient to the contaminants considered and suggests that some of the typical corticoles are more susceptible than others to levels of atmospheric pollutants. The most characteristic genera from this substrate *Macbrideola* and *Licea* (WRIGLEY de BASANTA 1998) and were much more numerous in the cultures from zone 1 and almost absent from the more polluted zone 4. For example 318 individual sporocarps of the very common species *Macbrideola cornea* were collected from the bark from zone 1 and not even one from zone 4. Even the cosmopolitan *Licea parasitica* was rare in zone 4 cultures. The widespread fructifications of *L. nannengae*, a species described

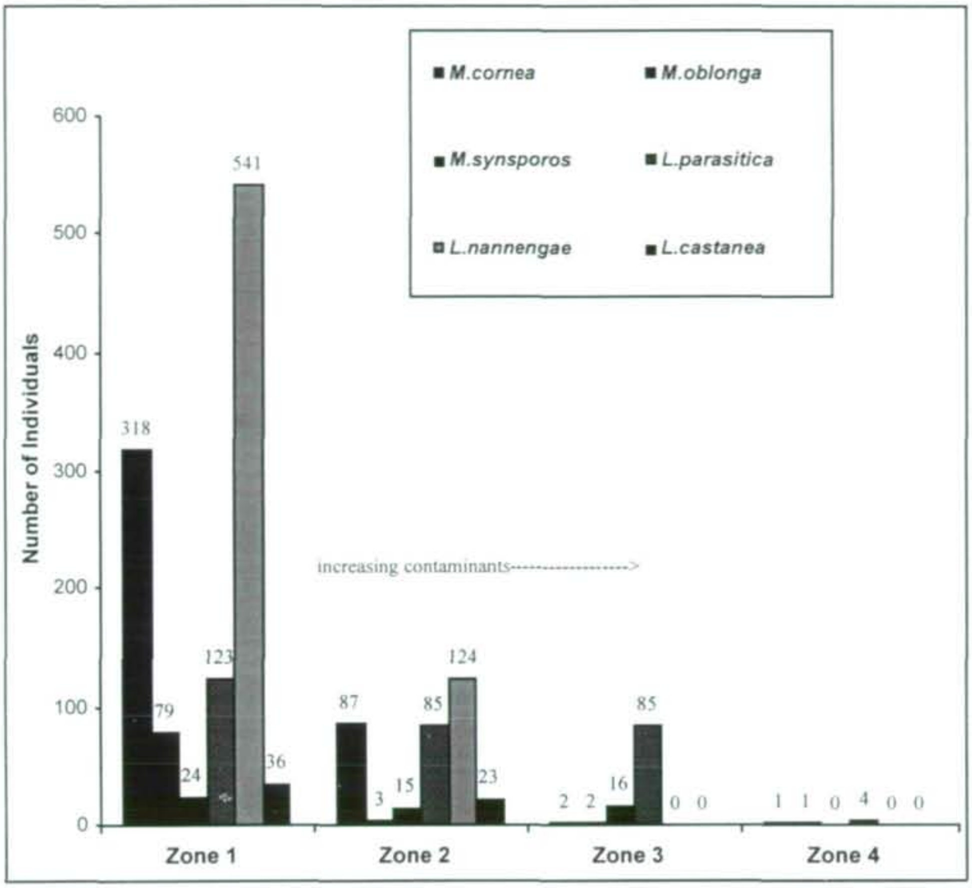

Fig. 4: Numbers of individuals of the most common species of myxomycetes from each zone.

by PANDO & LADO (1988) from Central Spain, show the most marked difference, with 541 sporocarps in total from the zone 1 cultures, fewer in zone 2 and none in zones 3 or 4. These species could constitute a group of very sensitive bioindicators, given their response to these contaminants. *Macbrideola synsporos* was found on bark from the first three zones and so seems less affected than *M. oblonga*, which was collected mostly from zone 1 bark. The two species of *Echinostelium* which appeared in these cultures, *E. minutum* and *E. apitectum* were almost all on bark from the centre of Madrid, the latter many times alongside *Arcyria cinerea*, which was only collected in these cultures on bark from zone 3. *E. apitectum* has been commonly found in Spain on *Olea europea* and *Juniperus thurifera* in the slightly acid pH range from 5.5-6.5 (PANDO 1989, PANDO & LADO 1990), and STEPHENSON (1989) found that *E. minutum* appeared on slightly acidic substrates. This suggests that they may be more acid tolerant than other species, but even so, none were found on bark from zone 4. The levels of SO^2 recorded here are far below those used by HAWKSWORTH & ROSE (1976) to relate particular lichen species to air quality zones. This may mean that myxomycetes are much more sensitive to, and therefore earlier indicators of, atmospheric contaminants than lichens. The samples in this study were collected and cultured at the same time and in the same way, so in spite of the modest number of cultures, the results are significant. They support the idea that there is a negative correlation between the number and variety of myxomycetes and the level of atmospheric contaminants. However, further cultures are needed to complete a detailed statistical analysis of the relationship.

Discussion

These results are in general agreement with those obtained by others (HÄRKÖNEN 1977; STEPHENSON 1988, 1989), and they are also consistent with those obtained by the author on oak bark in the UK, presented at ICSEM 1 (WRIGLEY de BASANTA unpublished data). They add support to the idea that pH plays a

role in determining the distribution of corticolous myxomycetes by the selection of tolerant species. Even with short term ameliorating conditions of 2 month cultures, irrigated with water at pH 6.8, some species will compete more aggressively with others which they exclude. This has been shown to occur on a far larger scale with lichens such as *Lecanora conizaeoides* which persists as the dominant species in the UK in species-poor communities in spite of lowering SO^2 levels (SEAWARD 1992).The repetition of the same few species in zones 3 and 4 in samplings 2 years apart would seem to support this. In the most contaminated areas the average number of records per culture (1-2) and the average number of species per culture (0.5-1.0) are similar to other results obtained from two different tree species from a contaminated area in the UK (WRIGLEY de BASANTA unpublished data). This is consistent with the idea that the species are selected for acid tolerance with few species surviving.

This effect on myxomycete distribution could be studied on a much larger scale by combining data already in existence of myxomycete collections from the bark of living trees and the existing SO^2 and NOx levels registered on country or continental maps (APSIMON et al. 1997; DOE 1993), in the same way as has been done for lichens (SEAWARD 1992). If long term forest health is to be sustained, recognition of all the components of the ecosystem is necessary, including the disruption to the community structure when pollution sensitive species are replaced by more tolerant ones (PETERS et al. 1996), altering population regulation of prey species.

Spain has the second highest forested area in the European Community (ASHMORE et al. 1990), covering 25% of its land mass, and protection of these forests is a top priority yet the impact of acid precipitation on Mediterranean forest ecosystems has hardly been studied (ASHMORE et al. 1990). Acid deposition on the Iberian Peninsula is only a recent problem. The European monitoring study (EMEP) shows that Spain still produces less SO^2 or NOx than Germany or the UK (APSIMON et al. 1997), and at present has many fewer damaged or unhealthy trees than the other European regions (ASHMORE et al. 1990). Madrid however is a rapidly growing city with frequent localised high levels of urban pollution. The buffering of the bark probably delays the effects of acid precipitation on the corticolous community, but this buffering potential is reduced by continued acid deposition, and the selection of more acid tolerant species of myxomycetes, suggested here, may be altering the population densities of other members of the microhabitat in these areas. Although the exact nature of their corticolous niche has still to be demonstrated, the obvious importance of the myxomycetes in food chain links should lead them to be considered as keystone species and to be used as a sensitive and simple bioindicator of forest health.

Acknowledgements

I am very grateful to Dr. Carlos LADO for reviewing material, for many discussions, for his help and his encouragement.

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